Practitioner's Docket No. U 012104-2 *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of
Inventor(s):

1. RAMA **MUKHERJEE**
2. MANU **JAGGI**
3. SUDHANAND **PRASAD**
4. ANAND C. **BURMAN**

WARNING: *37 CFR 1.41(a)(1) points out:*

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title):

NOVEL PEPTIDE ANALOGS FOR THE TREATMENT OF CANCER

CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date FEBRUARY 10, 1999, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EJ405329298US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

JENNIFER RASHKIN
(type or print name of person mailing paper)

Jennifer Rashkin
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C F R. 1 8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

WARNING: *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*

*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442*

1. Type of Application

This new application is for a(n)

(check one applicable item below)

[X] Original (nonprovisional)
[ ] Design
[ ] Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

[ ] Divisional.
[ ] Continuation.
[ ] Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copendng nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1 53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1 53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f)

37 CFR 1.78(a)(1).

NOTE If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S.*

application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application **must** be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

[ ] The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for Filing Date under 37 C.F.R. 1.53(b) (Regular) or 37 C.F.R. 1.153 (Design) Application

10 Pages of Specification
4 Pages of Claims
___ Sheets of Drawing
[ ] Formal
[ ] Informal

B. Other Papers Enclosed

1 Pages of Abstract
___ Other

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. 1.84, see Notice of March 9, 1988 . . (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page." 37 C.F.R. 1.84(c))

(complete the following, if applicable)

[ ] The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. 1.84(b).

4. Additional Papers Enclosed

[ ] Preliminary Amendment
[ ] Information Disclosure Statement (37 C.F.R. 1.98)
[ ] Form PTO-1449 (PTO/SB/08A and 08B)
[ ] Citations
[ ] Declaration of Biological Deposit
[ ] Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
[ ] Authorization of Attorney(s) to Accept and Follow Instructions from Representative
[ ] Special Comments
[ ] Other

5. Declaration or Oath

NOTE: A newly executed declaration is not required in a continuation or divisional application provided the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47 then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 CFR 1.63(d)

NOTE: A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and the residence, post office address and country of citizenship of each inventor and state whether the inventor is a sole or joint inventor 37 CFR 1.63(a)(1)-(4)

[ ] Enclosed
Executed by

(check **all** *applicable boxes)*

[ ] inventor(s).
[ ] legal representative of inventor(s). 37 CFR 1.42 or 1.43.
[ ] joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.
[ ] This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. See item 13 below for fee.

[X] Not Enclosed.

NOTE: *Where the filing is a completion in the U.S. of an International Application, or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

[X] Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 CFR 1.16(e),

can be filed subsequently).

NOTE: It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

[ ] Showing that the filing is authorized.
(not required unless called into question. 37 CFR 1.41(d))

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

[ ] The same.

or

[ ] Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,
[ ] is submitted.
[ ] will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 CFR 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 CFR 1.52(d).

[X] English
[ ] Non-English

[ ] The attached translation includes a statement that the translation is accurate. 37 C.F.R. 1.52(d).

8. Assignment

[X] An assignment of the invention to NATIONAL INSTITUTE OF IMMUNOLOGY AND DABUR RESEARCH FOUNDATION

[ ] is attached. A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ] FORM PTO 1595 is also attached.

[X] will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment" Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: *A newly executed "STATEMENT UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O G 62-64*

9. Certified Copy

Certified copy(ies) of application(s)

INDIA	UNKNOWN	FEBRUARY 11, 1998
Country	Appln. no.	Filed
Country	Appln. no.	Filed
Country	Appln. no.	Filed

from which priority is claimed

[ ] is (are) attached.
[X] will follow.

NOTE. The foreign applicaton forming the basis for the claim for priority must be referred to in the oath or declaration. 37 CFR 1.55(a) and 1.63

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U S APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. 1.16)

A. [X] Regular application

CLAIMS AS FILED

Claims	Number Filed	Basic Fee Allowance	Number Extra	Rate	Basic Fee 37 C.F.R. 1.16(a) $760.00
Total Claims (37 CFR 1.16(c))	31	-20 =	11	x 18	$ 198.00
Independent Claims (37 CFR 1.16(b))	15	- 3 =	12	x 78	936.00
Multiple Dependent Claim(s), if any (37 CFR 1.16(d))			+	$260.00	

[ ] Amendment cancelling extra claims is enclosed.
[ ] Amendment deleting multiple-dependencies is enclosed.
[X] Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $ 1,894.00

B. [ ] Design application ($310.00—37 CFR 1.16(f))

Filing Fee Calculation $_____

C. [ ] Plant application ($480.00—37 CFR 1.16(g))

Filing Fee Calculation $_____

11. Small Entity Statement(s)

[ ] Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 CFR 1.28(a)(2).*

(complete the following, if applicable)

[ ] Status as a small entity was claimed in prior application _____/_____, filed on _____ from which benefit is being claimed for this application under:

35 U.S.C. § [ ] 119(e),
[ ] 120,
[ ] 121,
[ ] 365(c),

and which status as a small entity is still proper and desired.

[ ] A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A**, **B** or **C** above) $_____

NOTE: Any excess of the full fee paid will be refunded if a small entity status is established refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1 136. 37 CFR 1.28(a).

12. Request for International-Type Search (37 C.F.R. 1.104(d))

(complete, if applicable)

[ ] Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

[X] Not Enclosed

[X] No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. 1.16(e) can be paid subsequently.)

[ ] Enclosed

[ ] Filing fee $_____

[ ] Recording assignment
($40.00; 37 C.F.R. 1.21(h))
(See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION.") $_____

[ ] Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached
($130.00; 37 C.F.R. 1.47 and 1.17(i)) $_____

[ ] For processing an application with a specification in a non-English language
($130.00; 37 C.F.R. 1.52(d) and 1.17(k)) $_____

[ ] Processing and retention fee
($130.00; 37 C.F.R. 1.53(d) and 1.21(l)) $_____

[ ] Fee for international-type search report
($40.00; 37 C.F.R. 1.21(e)) $_____

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 CFR 1.53(f) and this, as well as the changes to 37 CFR 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1 21(l) must be paid, within 1 year from notification under § 53(f)

Total Fees Enclosed $_____

14. Method of Payment of Fees

[ ] Check in the amount of $_____.

[ ] Charge Account No. _____ in the amount of $_____.
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should <u>not</u> be completed*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

[ ] The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No._____.

[ ] 37 C.F.R. 1.16(a), (f) or (g) (filing fees)

[ ] 37 C.F.R. 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action

[ ] 37 C.F.R. 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

[ ] 37 CFR 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).

[ ] 37 C.F.R. 1.17 (application processing fees)

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 CFR 1.136(a)(3).

[ ] 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1 311(b))

NOTE: 37 CFR 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . issue fee " From the wording of 37 CFR 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 CFR 1.26(a).

[ ] Credit Account No. ________________.

[ ] Refund

SIGNATURE OF PRACTITIONER

Reg. No. 33,778

JANET I. CORD
(type or print name of practitioner)

Tel. No.: (212)708-1935

LADAS & PARRY
P.O. Address
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023

Customer No.:

[ ] **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

[ ] Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added ___________

[ ] Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added ___________

[ ] Plus added pages deleting names of inventor(s) named on prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added ___________

[ ] Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added ___________

[ ] **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

[ ] This transmittal ends with this page.

NOVEL PEPTIDE ANALOGS FOR THE TREATMENT OF CANCER

FIELD OF THE INVENTION

This invention relates to novel peptide analogs of vasoactive intestinal peptide, somatostatin, bomebesin and Substance P. This invention also relates to the use of the novel peptide analogs for the treatment of cancer.

BACKGROUND OF THE INVENTION

Neuropeptide analogs are increasingly used in the treatment of cancer. The neuropeptides vasoactive intestinal peptide and bombesin exert physiological effects by binding to specific receptors present on cells in the gastrointestinal tract and central nervous system.

Bombesin is an amphibian peptide that has a structure closely related to that of several mammalian peptides, including gastrin releasing peptide (GRP) and Neuromedins B and C. Bombesin was discovered in 1970 and is a potent smooth muscle contracting agent of nonmammalian origin first isolated from amphibian skin (Erspamer et al. J. Pharm. Pharmacol. 22:275 (1970)). Bombesin, GRP and related peptides exert their *in vivo* effects by binding to specific receptors present on cells of the gastrointestinal tract, the central nervous system and on tumors.

VIP is a 28-amino acid neuropeptide, which has been implicated as a major growth promoting factor during embryonic growth. In cancer cells, previous studies have implied that VIP can serve as an autocrine growth factor in lung tumors (Gozes et al. Biomed. Res. 13 (Suppl. 2) 37, (1992)). By blocking the binding of VIP to its receptor these analogs inhibit the growth of tumor cells that respond to the growth-promoting action of VIP.

In U.S. Patent Application 08/727,679, we have described the role of neuropeptides in cancer. High affinity and moderate affinity receptors for vasoactive intestinal peptide and somastostatin, high affinity receptors for bombesin and moderate affinity receptors for substance P were demonstrated on human colon adenocarcinoma cells. It was further demonstrated that peptide analogs to the above neuropeptides could actively and selectively induce cell death in the cancer cells. A formulation of peptide combination termed MuJ-7 has also been described which

causes tumor regression in xenotransplanted nude mice. The individual constituent peptides of MuJ-7 were demonstrated to have anticancer activity.

SUMMARY OF THE INVENTION

This invention provides novel peptides. These peptides are useful for the treatment of cancer. The invention relates to novel bombesin analogs which act as antagonists of bombesin or related peptides such as gastrin releasing peptide. By blocking the binding of bombesin-like peptides to their receptors, these antagonists block the physiological effects of these peptides and inhibit the growth of tumor cells that respond to growth-promoting action of bombesin. Thus these antagonists have therapeutic use in the treatment or prevention of cancer and in controlling physiological effects in gastrointestinal disorders and in modulating responses of the central nervous system.

The invention also relates to novel vasoactive intestinal peptide analogs which act as antagonists of VIP by blocking the binding of VIP to its cognate receptors.

The invention further relates to the designing and testing of novel structural analogs of Substance P and somatostatin, and analogs of VIP receptor binding inhibitor and bombesin antagonist which have been designed to render conformational constraints and higher stability to the peptides while maintaining their anticancer activity. Substitution and/or deletions have been incorporated into for example, VIP_2 and BOM_1 sequences bearing in mind not to alter amino acids known to offer conformational constraint and stability to the peptide. In order to introduce conformational constraints, unusual amino acids such as cyclic and acyclic dialkylated glycines have been incorporated into the peptide backbone. Preferably the cyclic ring is a C_3-C_8 ring and the number of carbon atoms in the alkyl group is from 1 to 6 (methyl to hexyl). Examples of such amino acids are Aib, MeLeu, Di-ethylglycine and its higher homologs, and 1-amino cycloalkane carboxylic acids. Aib represents α-amino-isobutyric acid.

DETAILED DESCRIPTION OF THE INVENTION

The VIP receptor binding inhibitor VIP_2 (Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys) (SEQ ID NO:1) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for vasoactive intestinal peptide.

Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys with Dxg. Dxg represents cyclic and acyclic dialkylated glycines where the cyclic ring is a C_3-C_8 ring and the number of carbon atoms in the alkyl group is from 1 to 6 (methyl to hexyl). Examples are Aib, MeLeu, Di-ethylglycine and its higher homologs, and 1-amino cycloalkane carboxylic acids. Aib represents α-amino-isobutyric acid.

Novel peptides include:

DT-11 Aib-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO:2)

DT-12 D-Leu-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO:3)

DT-13 Leu-Met-Tyr-Pro-Thr-D-Tyr-Leu-Lys-OH (SEQ ID NO:4)

DT-14 Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO:5)

DT-15 Leu-Met-D-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO:6)

DT-16 D-Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO:7)

DT-18 Aib-Met-Tyr-Pro-Thr-Tyr-Dxg-Lys-OH (SEQ ID NO:8)

DT-19 D-Leu-Met-Tyr-Pro-Thr-Tyr-Dxg-Lys-OH (SEQ ID NO:9)

where Dxg and Aib are as defined above.

The bombesin antagonist BOM_1 (D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NHEt) (SEQ ID NO:10) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for bombesin. Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NHEt (SEQ ID NO:10) with Dxg where Dxg is as defined above. Leucine may be replaced with isoleucine and tryptophan may be replaced by D-tryptophan.

Novel peptides include:

DT-21 D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH_2 (SEQ ID NO:11)

DT-22 D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID

NO:12)

DT-23 D-Phe-Gln-Trp-Aib-Val-Gly-His-Leu-NH_2 (SEQ ID NO:13)

DT-24 D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO:14)

DT-25 D-Phe-Gln-Trp-Ala-Val-Gly-His-Ile-NH_2 (SEQ ID NO:15)

DT-26 D-Phe-Gln-Trp-Aib-Val-Gly-His-Ile-NH_2 (SEQ ID NO:16)

DT-27 D-Phe-Gln-Trp-Ala-Val-Aib-His-Ile-NH_2 (SEQ ID NO:17)

wherein Aib represents alpha-amino isobutyric acid.

The Substance P analog (D-Arg-Pro-Lys-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 (SEQ ID NO:18)) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for Substance P. Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence D-Arg-Pro-Lys-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 (SEQ ID NO:18)) with Dxg or Aib. Dxg and Aib are as defined above. Analogs may be 5 to 11 amino acids.

Novel peptides include:

DT-31 Aib-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:19)

DT-32 Dxg-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:20)

DT-33 D-Leu-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:21)

DT-34 D-Arg-Pro-Lys-Pro-Aib-Gln-D-Trp-Phe-D-Trp-Aib-Leu-NH_2 (SEQ ID NO:22)

DT-35 Arg-Pro-Aib-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 (SEQ ID NO:23)

where Dxg and Aib are as defined above.

The somatostatin analog (Ala-Gly-Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys (disulfide bridges: 3-14) (SEQ ID NO:24) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for Somatostatin. Novel peptides that have conformational constraints and

resist enzymatic degradation are formed by replacing any of the amino acids of the sequence (Ala-Gly-Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys (disulfide bridges:3-14) (SEQ ID NO:24) with Dxg or Aib where Dxg and Aib are as defined above.

A novel peptide is:

DT-61 Ala-Aib-Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-D-Ser-Cys (3-14 disulfide bond) (SEQ ID NO:25)

where Aib represents α-amino isobutyric acid.

The somatostatin analog (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2) (SEQ ID NO:26) has been shown in our previous studies to be a selective cytotoxic peptide for cancer cells having receptors for Somatostatin. Novel peptides that have conformational constraints and resist enzymatic degradation are formed by replacing any of the amino acids of the sequence D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (SEQ ID NO:26) with Dxg or Aib where Dxg and Aib are as defined above.

A novel peptide is:

DT 71 D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Aib-Thr-NH_2 (SEQ ID NO:27)

where Aib represents alpha-amino isobutyric acid.

The present invention is further described in detail with reference to the following examples, which are given for purpose of merely illustrating the invention without limiting it.

EXAMPLE 1

The cytotoxic activity of the peptides synthesized was tested on eight human tumor cell lines namely HT-29, SW620, PTC (all colon), PA-1 (ovary), A549 (lung), HBL100 (breast), MOLT-4 (leukemia) and DU145 (prostate). The tumor cells were collected at exponential growth phase and resuspended in medium (1.5×10^6 cells/ml in RPMI 1640 containing 10% FBS). 150 µl of medium was added to the wells of a 96-well tissue culture plate (Nunc, Denmark) followed by 30 µl of cell suspension. The plate was left in an incubator (37°C, 5% CO_2) overnight. 20 µl of the pepide (10^{-7} to 10^{-10}M concentration) was added to marked wells of the 96 well plate. Each concentration was plated in triplicate. 20 µl of medium alone was added to control wells while wells without cells served as blanks. A total volume of 200 µl

was ensured in each well and the plate was left in the incubator (37°C, 5% CO_2). After 72 hours of incubation an MTT assay was performed and percentage cytotoxicity was calculated with respect to control cells. DT-1: bombesin antagonist, DT-2: VIP receptor binding inhibitor, DT-3: substance P analog, DT-4 and DT-7: Somastatin analogs.

TABLE 1

PERCENT CYTOTOXICITY OF DT-1 ANALOGS ON VARIOUS TUMOR CELL LINES

Cell line	DT11	DT12	DT13	DT14	DT15	DT16	DT18	DT19
PA-1	32	26	23	14	25	22	31	34
PCT	16	24	20	0	23	18	14	10
MOLT-4	30	42	39	30	33	31	36	23
HBL100	24	19	27	19	19	2	24	27
A549	29	36	32	42	37	33	27	25
SW620	13	0	18	12	28	22	26	33
HT29	0	7	6	6	11	20	38	30
DU145	7	19	1	5	10	0	18	24

TABLE II

PERCENT CYTOTOXICITY OF DT-2 ANALOGS
ON VARIOUS TUMOR CELL LINES

Cell line	DT21	DT22	DT23	DT24	DT25	DT26	DT27
PA-1	23	24	16	33	21	24	26
PTC	18	12	15	23	9	25	12
MOLT-4	25	17	29	8	23	20	13
HBL100	27	14	33	32	25	6	16
A549	16	22	23	30	25	13	18
SW620	25	33	34	38	31	38	32
HT29	24	35	43	44	40	27	28
DU145	10	22	25	32	33	24	0

TABLE III

PERCENT CYTOTOXICITY OF DT-3 ANALOGS
ON VARIOUS TUMOR CELL LINES

Cell line	DT31	DT32	DT33	DT34	DT35
PA-1	24	ND	ND	ND	ND
PTC	21	32	23	16	18
MOLT-4	30	32	37	26	20
HBL100	15	16	15	21	15
A549	23	19	21	24	20
SW620	14	ND	ND	ND	ND
HT29	30	13	18	9	26
DU145	25	ND	ND	ND	ND

TABLE IV

PERCENT CYTOTOXICITY OF DT-6 ANALOGS
ON VARIOUS TUMOR CELL LINES

Cell line	DT61
PA-1	45
PTC	22
MOLT-4	34
HBL100	26
A549	28
SW620	26
HT29	35
DU145	29

TABLE V

PERCENT CYTOTOXICITY OF DT-7 ANALOGS
ON VARIOUS TUMOR CELL LINES

Cell line	DT71
PA-1	18
PTC	19
MOLT-4	37
HBL100	23
A549	18
SW620	ND
HT29	19
DU145	ND

EXAMPLE 2

A 0.5 mL of 2000 ppm of VIP_2 was mixed with 1.0 ml of freshly prepared liver homogenate to obtain a concentration of 1000 ppm. Sample preparations were incubated at 37°C and after time intervals of 0, 2, 5, 10, 20 and 30 minutes, 200 µl of the preparation was aliquoted and precipitated with equal volumes

of acetonitrile. In case of BOM_1 analogs, the sample preparations with a final concentration of 1000 ppm were incubated at 37°C and after time intervals of 0, 15, 30, 60, 90, 120 and 150 minutes, 200 µl of the preparation was aliquoted and precipitated with equal volumes of acetonitrile. The precipitate was pelleted by centrifugation at 10,000 g for 5 minutes and supernatant was analyzed by HPLC. The percentage increase in the half-life of DT1 (VIP receptor binding inhibitor) and DT2 (bombesin antagonist) analogs with reference to DT1 and DT2 respectively, as estimated by the mouse liver homogenate study is shown in Tables VI and VII respectively.

TABLE VI

HALF LIFE OF DT-1 ANALOGS WITH REFERENCE TO DT-1 AS DETERMINED BY THE MOUSE LIVER HOMOGENATE STUDY

Peptide	Half-life (minutes)
DT-1	4.9
DT-11	15.4
DT-12	18.1

TABLE VII

HALF LIFE OF DT-2 ANALOGS WITH REFERENCE TO DT-2 AS DETERMINED BY THE MOUSE LIVER HOMOGENATE STUDY

Peptide	Half-life (minutes)
DT-2	12.6
DT-22	15.06
DT-23	57.7
DT-24	38.5
DT-26	114.5
DT-27	292.5

PREPARATION OF MOUSE LIVER HOMOGENATE

1. Healthy Balb/c mouse was sacrificed and dissected to expose liver.
2. The pulmonary artery was severed to drain blood and cold saline

was perfused through the portal vein until the liver becomes pale white.

3. The liver was excised, minced and passed through 60# sieve.

4. 1.15% w/v KCl -0.01M phosphate buffer (pH 7.4) was added to make 20% w/v homogenate tht was centrifuged at 4500 g for 15 min.

5. The supernatant was recovered and further centrifuged at 10,000 g to clarify the homogenate.

CLAIMS

1. A peptide of the sequence Leu^1-Met^2-Tyr^3-Pro^4-Thr^5-Tyr^6-Leu^7-Lys^8 wherein at least one of the amino acids at positions 1-8 is replaced by Dxg.

2. A peptide having the sequence selected from the group consisting of:
Aib-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO: 2);
D-Leu-Met-Tyr-Pro-Thr-Tyr-Aib-Lys-OH (SEQ ID NO: 3);
Leu-Met-Tyr-Pro-Thr-D-Tyr-Leu-Lys-OH (SEQ ID NO: 4);
Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO: 5);
Leu-Met-D-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO: 6);
D-Leu-Met-Tyr-Pro-Thr-Tyr-D-Leu-Lys-OH (SEQ ID NO: 7);
Aib-Met-Tyr-Pro-Thr-Tyr-Dxg-Lys-OH (SEQ ID NO: 8); and
D-Leu-Met-Tyr-Pro-Thr-Tyr-Dxg-Lys-OH (SEQ ID NO: 9).

3. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 1 to a patient in need thereof.

4. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 2 to a patient in need thereof.

5. A peptide of the sequence D-Phe^1-Gln^2-Trp^3-Ala^4-Val^5-Gly^6-His^7-Leu^8-NHEt wherein at least one of the amino acids at positions 1-8 is replaced by Dxg.

6. A peptide of the sequence D-Phe-Gln-Trp-Ala-Val-Gly-His-Ile-NHEt (SEQ ID NO: 28).

7. A peptide of the sequence D-Phe-Gln-D-Trp-Ala-Val-Gly-His-Leu-NHEt (SEQ ID NO: 29).

8. A peptide according to claim 5, wherein Leu is replaced by Ile, Trp is replaced by D-Trp or a combination thereof.

9. A peptide selected from the group consisting of:
D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH_2 (SEQ ID NO: 11);
D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 12);
D-Phe-Gln-Trp-Aib-Val-Gly-His-Leu-NH_2 (SEQ ID NO: 13);

D-Phe-Gln-Trp-Ala-Val-Aib-His-Leu-NH_2 (SEQ ID NO: 14);

D-Phe-Gln-Trp-Ala-Val-Gly-His-Ile-NH_2 (SEQ ID NO: 15);

D-Phe-Gln-Trp-Aib-Val-Gly-His-Ile-NH_2 (SEQ ID NO: 16); and

D-Phe-Gln-Trp-Ala-Val-Aib-His-Ile-NH_2 (SEQ ID NO: 17);

wherein Aib represents alpha-amino isobutyric acid.

10. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 5 to a patient in need thereof.

11. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 9 to a patient in need thereof.

12. A peptide of the sequence D-Arg^1-Pro^2-Lys^3-Pro^4-D-Phe^5-Gln^6-D-Trp^7-Phe^8-D-Trp^9-Leu^{10}-Leu^{11}-NH_2 wherein at least one of the amino acids at positions 1-11 is replaced by Dxg.

13. A peptide of the sequence D-Arg^1-Pro^2-Lys^3-Pro^4-D-Phe^5-Gln^6-D-Trp^7-Phe^8-D-Trp^9-Leu^{10}-Leu^{11}-NH_2 wherein at least one of the amino acids at positions 1-11 is replaced by Aib.

14. A peptide according to claim 12, wherein the sequence comprises 5 to 11 amino acids.

15. A peptide according to claim 13, wherein the sequence comprises 5 to 11 amino acids.

16. A peptide selected from the group consisting of:

Aib-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:19);

Dxg-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:20);

D-Leu-Met-Gln-Trp-Phe-Aib-NH_2 (SEQ ID NO:21);

D-Arg-Pro-Lys-Pro-Aib-Gln-D-Trp-Phe-D-Trp-Aib-Leu-NH_2 (SEQ ID NO:22); and

Arg-Pro-Aib-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 (SEQ ID NO:23).

17. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 12 to a patient in need thereof.

18. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 13 to a patient in need thereof.

19. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 16 to a patient in need thereof.

20. A peptide of the sequence Ala^{1}-Gly^{2}-Cys^{3}-Lys^{4}-Asn^{5}-Phe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}-Phe^{11}-Thr^{12}-Ser^{13}-D-Cys^{14} (disulfide bridges:3-14) wherein at least one of the amino acids at positions 1-14 is replaced by Dxg.

21. A peptide of the sequence Ala^{1}-Gly^{2}-Cys^{3}-Lys^{4}-Asn^{5}-Phe^{6}-Phe^{7}-D-Trp^{8}-Lys^{9}-Thr^{10}-Phe^{11}-Thr^{12}-Ser^{13}-D-Cys^{14} (disulfide bridges:3-14) wherein at least one of the amino acids at positions 1-14 is replaced by Aib.

22. A peptide of the sequence Ala-Aib-Cys-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-D-Ser-Cys (3-14 disulfide bond) (SEQ ID NO:25).

23. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 20 to a patient in need thereof.

24. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 21 to a patient in need thereof.

25. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 22 to a patient in need thereof.

26. A peptide of the sequence D-Phe^{1}-Cys^{2}-Tyr^{3}-D-Trp^{4}-Orn^{5}-Thr^{6}-Pen^{7}-Thr^{8}-NH_2 wherein at least one of the amino acids at positions 1-8 is replaced by Dxg.

27. A peptide of the sequence D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 wherein at least one of the amino acids at positions 1-8 is replaced by Aib.

28. A peptide of the sequence D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Aib-Thr-NH_2 (SEQ ID NO:27).

29. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 26 to a patient in need thereof.

30. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 27 to a patient in need thereof.

31. A method for treating colon, breast, ovarian, lung, or prostate cancer or leukemia comprising administering an effective amount of a peptide according to claim 28 to a patient in need thereof.

ABSTRACT

This invention relates to novel peptide analogs of vasoactive intestinal peptide, somatostatin, bombesin and Substance P. This invention also relates to the use of the novel peptide analogs for the treatment of cancer.